Synopsis for Ph.D Programme

Bio-hydrogen production from lignocellulosic biomass through dark fermentation process

Prepared by

Pushpa Rani

Supervisor

Prof. Narsi Ram Bishnoi

Department of Environmental Science & Engineering,

Guru Jambheshwar University of Science & Technology,

Hisar-125001(Haryana)

INTRODUCTION

The environmental and global warming consciousness has become an important policy in all countries around the world. Furthermore, the fossil fuel use has been related to some alarming environmental problems such as global warming and climate change (Tsai et al, 2016 and Vu et al, 2017). Today, fossil fuel including oil, natural gas, and coal provide 32.6%, 23.7% and 30% of the total world energy consumption and are the primary sources of the global energy supply (BP, 2015). Technology enhancements and human development contribute to the continuous increase in the worldwide energy demand (Okonko et al, 2009). In spite of the extensive utilization and application of fossil resources, these are non-renewable and will not last forever. Oil, natural gas and coal supplies will be depleted in about 45, 60, and 120 years, respectively (Alavijeh & Yaghmaei, 2016). Due to depleting petroleum reserves and the environmental impact of fossil fuels, it is important to find alternative sources for transport fuels (Kasmuri et al, 2017). Switching from fossil fuels to renewable energy could lead to positive impacts for most countries in the world (Sopian et al, 2010) . Hydrogen gas (H_2) is a promising alternative energy carrier due to its significant properties such as high calorific value (142 KJ/g), CO_2 -neutral, and renewable (Zhang et al, 2011; Lee et al, 2012; Arizzi et al, 2016 and Sivagurunathan et al, 2017). Efficient methods of hydrogen generation from renewable sources, including waste products, are currently being developed, even though hydrogen is mainly produced through steam reforming or thermal cracking of natural gas or petroleum fractions (Lukajtis et al, 2018).

Among the H_2 production processes, biological H_2 production is a promising alternative. Bio-hydrogen can be produced from various feed-stocks like municipal solid wastes, waste-water sludge, lignocellulosic wastes and industrial wastes (Singhal et al, 2012 and Jeihanipour and Bashiri, 2015). To reduce the production cost, it is preferable to produce biohydrogen from lignocellulosic (LC) biomass, which are the most abundant, less expensive and renewable resource available in nature that do not compete with food crops. Thus, LC biomass signifies a huge potential in terms of renewable energy and has drawn tremendous amount of attention for the conversion of LC biomass to biofuels (Lo et al, 2011; Saratale et al, 2013). Lignocellulosic wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicellulose and lignin. They may be grouped into different categories such as wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, stover, peelings, cobs, stalks, nutshells, non food seeds, bagasse)

domestic wastes (lignocellulosic garbage and sewage) and food industry residues (Adeniran et al, 2017).

Bioconversion of lignocellulosic biomass to fermentable sugars requires three sequential steps: pretreatment, hydrolysis and fermentation (Singh et al, 2011). To enhance the digestibility of cellulose-based biomass, pretreatment methods are required to remove or solubilize the lignin present in cellulose-based biomass. Besides, pretreatment is also beneficial in reducing the crystallinity of cellulose, enlarging the available surface area and further enhance the biodegradability of cellulose-based biomass. Thus, various physical, chemical, physicochemical and biological technologies have been explored for biomass pretreatment taking into account of enhancing the yields of fermentable sugars and subsequently biohydrogen production using dark fermentation strategies (Saratale et al, 2018).

Alternatively, hydrogen can be produced from biomass using natural capabilities of microorganisms to generate hydrogen as one of the metabolic products. There are two methods to produce H_2 from microorganisms. The first method uses photosynthetic micro-organisms such as bacteria or algae (photofermentative processes) and the second method uses fermentative organisms (dark fermentation processes) (Ren et al, 2009). Dark fermentative biohydrogen production has received considerable attention because of the potential utilization of a wide variety of carbohydrate-rich wastes, lower operational costs, higher efficiency, simpler control requirements and considerable role in waste reduction. Dark fermentation is also the best understood process among biotechnological methods of hydrogen production (Manish and Banerjee, 2008). Biohydrogen production using dark fermentation can be performed by either pure cultures or anaerobic microbial consortia. Mixed anaerobic consortia are usually preferred because of potential expression of a wide range of hydrolytic activities to enhance substrate utilization especially for complex lignocellulosic compounds, no need to medium sterilization, simpler control and operation and more robust to changes in the environmental conditions such as pH and temperature (Zilouei and Taherdanak, 2015).

Hydrogen producing bacteria occurring in nature do not exist in isolation. They often live in the presence of other microorganisms, mainly archaea. Mixed cultures in addition to hydrogen-producing microorganisms also contain bacteria consuming hydrogen in their metabolic

processes. These are mainly methanogens and homoacetogens as well as bacteria reducing sulfur, producing propionic acid and reducing nitrates consuming hydrogen in their metabolic processes. This requires special pretreatment of an inoculum to partly or completely inactivate the microorganisms responsible for hydrogen consumption (Lukajtis et al, 2018).

Based on the above background, proposed work is planned for management of lignocellulosic waste and its conversion to Hydrogen. Main focus of work is to reduce cost and enhance efficiency.

OBJECTIVES:

- Screening, characterization and pretreatments of lignocellulosic biomass.
- Isolation and screening of microbial consortium for hydrogen production.
- Nutritional and operational parameters optimization for hydrogen production.
- Bio-hydrogen production in lab scale bioreactor under optimized conditions.

REVIEW OF LITERATURE:

The literature review in this particular area pertains to different strategies followed to produce biohydrogen from lignocellulosic waste worldwide. However, the scope of this work is to enhance efficiency and to reduce cost of biohydrogen production. Kotay & Das (2008) discussed that Biohydrogen is considered as an important key to a sustainable world power supply and is currently being seen as the versatile fuel of the future, with the potential to replace fossil fuels. It has the key prospective to become the ideal means among the range of renewable H_2 production technologies presently existing. Monlau et al (2012) analysed compositional (lignin, cellulose, hemicelluloses, total uronic acids, proteins, and soluble sugars) as well as structural features (crystallinity) for 20 lignocellulosic residues. Kumari & Singh (2018) discussed and compared different pretreatment methods, biomass resources, chemical composition of different agricultural biomass and the use of this biomass for bioenergy generation. Various pretreatment processes used for bio-hydrogen, bio-methane, bio-ethanol, bio-methanol bio-butanol and bio-diesel production are also discussed. Dong et al (2018) studied different alkaline solutions with both presence and absence of urea and analysed the maximum hydrogen production and energy conversion efficiency of 22.08 mmol/L and 9.76% were obtained from NaOH/Urea pretreated

rice straw at low temperature. Zhao et al (2012) investigated fungal pretreatment of cornstalk by *Phanerochaete chrysosporium* and observed lignin reduction up to 34% and the pretreated cornstalk was subjected to enzymatic hydrolysis. The maximum enzymatic saccharification was found to be 20.3% higher than the control without pretreatment.

Gonzales et al (2017) investigated dark fermentation of rice husk .The substrate was pre-treated with 5% sulfuric acid at 60^{o}C followed by enzymatic sacharification with varying concentrations and the highest obtained hydrogen yield was equal to 473.1 cm3 H2/g rice husk. Jiang et al (2015) evaluated thermophilic hydrogen fermentation of cellulose by a long term continuous experiment and batch experiments. Lo et al (2011) have observed that the hydrolysates carboxymethyl-cellulose (CMC), xylan, and bagasse were efficiently converted to H_2 via dark fermentation with *Clostridium butyricum CGS5*. Guo et al (2010) reported biohydrogen production from agricultural wastes by dark fermentation and key operational parameters such as pH, partial pressure, temperature and discussed to microbial factors facilitate further research in this domain. Sivagurunathan et al (2017) analyzed selected fermentative biohydrogen production processes by revisiting the core biohydrogen production performances in terms of gas production rates and yields and equally addressesed the options for process enhancement by the application through pretreatment methods and detoxification of process inhibitors.

Guo et al (2016) majorly focused on pretreatment methods, hydrogen-producing microorganisms and process engineering. Łukajtis et al (2018) compiled essential information on strains of bacteria used in the production of hydrogen from waste products in the agro-industry and from lignocellulosic biomass. The effect of such parameters as kind of raw material, method of processing, temperature, pH, substrate concentration, partial pressure of hydrogen, hydraulic retention time, method of inoculum preparation and the type and operating parameters of a reactor on the yield of dark fermentation was discussed. Rabelo et al (2018) investigated the individual and mutual effects of substrate concentration (from 0.8 to 9.2 g/L) and pH (from 4.6 to 7.4) on hydrogen and volatile fatty acids production from sugarcane bagasse (SCB) in batch reactors. Reginatto, & Antônio (2015) compared competitive biological hydrogen production from lignocellulosic materials without any type of pretreatment and lignocellulosic materials after a pretreatment step. The average H_2 production from pretreated material was 50% higher as compared with the average yield achieved using untreated lignocellulosic materials.

RESEARCH METHODOLOGY:-

Collection, characterization and pretreatment of lignocellulosic waste

- Lignocellulosic waste will be collected and screened depending upon the local availability.
- Characterizations of waste samples will be done using standard methods.
- Pretreatment of lignocellulosic waste will be done with the acid and alkali to enhance efficiency of hydrogen production.

Isolation and screening of microbial consortium for hydrogen production

- Microbial consortium will be isolated from various sites, such as distillery industry / landfill site / dumpsite etc.The methanogenic microbes will be killed first from the samples.So that only hydrogen producing microbes can grow properly and produce hydrogen.

Nutritional and operational parameters optimization for hydrogen production.

- Different parameters like temperature, pH, incubation time, substrate concentration, nitrogen etc. will be optimized for high production of hydrogen.

Bio-hydrogen production in lab scale bioreactor under optimized conditions.

- Evaluation of hydrogen production under optimized conditions will be done in lab scale bioreactor.

YEARLY WORK PLAN:

1st year plan

- Literature review.
- Collection of lignocellulosic waste samples.
- Characterization and pretreatment of lignocellulosic waste

2nd year plan

- Screening and Isolation of microbial consortia collected from various site.
- Microbes except hydrogen producing will be killed by heating.
- Optimization of nutritional and operational parameters for hydrogen production.

3rd year plan

- Production of bio-hydrogen from LC biomass through hydrogen producing bacteria.
- Compilation of research findings and interpreting results
- Thesis writing

SCOPE OF THE STUDY: -

Continuous depletion of fossil fuels, their increasing price, environmental and ecological point of views have stimulated the reawakened interest in developing sustainable and renewable energy sources to lessen the reliance on fossil fuels. To reduce the production cost, it is preferable to produce biohydrogen from lignocellulosic (LC) biomass, which are the most abundant, less expensive and renewable resource available in nature that do not compete with food crops. Thus LC biomass signifies a huge potential in terms of renewable energy and has drawn tremendous amount of attention for the conversion of LC biomass to biofuels. Hydrogen production by conversion of lignocellulosic biomass is both sustainable and environmentally friendly, which has been paid more and more attention all over the world. According to the present relevant studies, the bio-hydrogen production fermentation processes can be classified into photosynthetic fermentation process and anaerobic dark fermentation process .In comparison these two processes, anaerobic fermentation is more advantageous because of its easier operation and control system, lower energy consumption, higher hydrogen production rate and broader substrates, such as abundant and renewable biomass wastes. For conversion of lignocellulosic biomass into hydrogen, lignin should be decomposed by pretreatment processes at first (such as acid hydrolysis, and alkali), then fermented by microorganisms which can directly utilize cellulose to produce hydrogen. The inoculum source chosen in most of the studies have been a mixed culture. Physicochemical methods for H_2 production produce a mixture of gases (H_2, CH_4, CO_2, CO and N_2), require high temperatures (>850^oC), derived from combustion of fossil fuels, there by being energy intensive and expensive. To overcome these problems lignocellulosic residues, has gained much interest for Hydrogen production. It is an inexpensive, renewable,

abundant and provides a unique natural resource. Now-a-days physicochemical methods are used for conversion to Hydrogen but they are energy intensive methods and offer high operating as well as capital costs, so an environment friendly process is required to overcome these challenges. One such cost-effective method is Hydrogen production via biological method. The aim of the my research work is to develop an alternative renewable, sustainable and cost-effective green technology for production of hydrogen by means of biological conversion process of LC waste.

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